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HEMOSTATIC ACTIVITY OF CHITOSAN IN WOUND MANAGEMENT

Technical Progress Report No. 1
For the Period January 1, 1989 to March 31, 1989

Prepared Under
Contract No. N00014-89-C-0024

MARCH, 1989

Prepared for
OFFICE OF NAVAL RESEARCH
Department of the Navy
Arlington, VA 22217

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Unclassified
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION Unclassified	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for Public Release Distribution Unlimited
2b DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Technical Progress Report No. 1	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION 3M Wound Management Products Laboratory 6b OFFICE SYMBOL (*If applicable*)	7a NAME OF MONITORING ORGANIZATION Office of Naval Research
6c ADDRESS (*City, State, and ZIP Code*) Building 270-4N-03 3M Center St. Paul, MN 55144-1000	7b ADDRESS (*City, State, and ZIP Code*) Department of the Navy 800 N. Quincy Street Arlington, VA 22217
8a NAME OF FUNDING / SPONSORING ORGANIZATION 8b OFFICE SYMBOL (*If applicable*)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. N00014-89-C-0024
8c ADDRESS (*City, State, and ZIP Code*)	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO / PROJECT NO / TASK NO / WORK UNIT ACCESSION NO

11 TITLE (*Include Security Classification*)
Hemostatic Activity of Chitosan in Wound Management

12 PERSONAL AUTHOR(S)
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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (*Year, Month, Day*)	15 PAGE COUNT
Quarterly	FROM Jan 1, 89 TO Mar 31 89	89 Mar 27	5

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	Collagen Chitosan Glutamate Hemostatic Agent

19 ABSTRACT (*Continue on reverse if necessary and identify by block number*)

Research has been initiated on the preparation of a hemostatic agent which contains chitosan glutamate. A source for chitosan glutamate raw material has been located and characterization of the material is underway. A source for the collagen portion of the composite hemostatic sponge has also been identified and prototype sponges are currently being produced both by 3M and an outside vendor. The preclinical testing plan has been reviewed with the FDA, and it appears at this stage that the testing plan, as proposed, is an appropriate starting point.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified
22a NAME OF RESPONSIBLE INDIVIDUAL L.C. McGraw	22b TELEPHONE (*Include Area Code*) (612) 733-9816 — 22c OFFICE SYMBOL

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

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1.0 INTRODUCTION

This report covers progress during the first through third months of the Office of Naval Research Contract N00014-89-C-0024. The various sections of the report are numbered and titled using the format of the original proposal.

2.0 PROGRESS REPORT

2.1 Task 1: Raw Material Characterization

During the past quarter, suppliers and appropriate purity grades have been identified for both chitosan glutamate and collagen, the raw materials necessary for production of the hemostatic product. Efforts have been initiated toward the complete analytical characterization of the chitosan glutamate; it appears that the collagen will be adequately certified by the vendor.

2.1.1 Subtask 1.1: Quality Assurance Testing

Protan, Inc. has developed, in conjunction with 3M, an ultrapurified grade of several chitosan salts designated Protosan LV. One lot (no. 808-572-01) of this ultrapure chitosan glutamate has been ordered and received. The following analytical information was provided with the material.

Particulates:	Solution filtered through < 5 micron filter	
Viscosity:	92 cps (1% solution, 20°C)	
Solution pH:	5.0	
Metal content:	Na	125 ppm
	Ca	16 ppm
	K	8 ppm
	Mg	4 ppm
	Zn	20 ppm
	Fe	80 ppm
Percent Deacetylation:	79	

A sample of this lot of chitosan glutamate has been submitted to 3M Analytical Research for additional heavy metal analysis and elemental analysis. Results will be available next quarter.

Previous work in our laboratory had identified uv spectroscopic methods for the determination of the percent deacetylation of chitosan free base dissolved in dilute acetic acid (1). Since the chitosan is now available in the purified salt form, methods are being developed to measure the degree of deacetylation without precipitating the free base. Preliminary experiments suggest that a slightly modified uv procedure will be acceptable for the direct determination of the chitosan glutamate as received from the vendor. The vendor's analysis will be verified until vendor reliability has been established.

Semex Medical has been identified as a source for the collagen portion of the hemostatic agent that will serve as the carrier for the chitosan glutamate. This vendor offers exceptionally high purity collagen extracted from bovine hides. Semed S (TM) is a water soluble mixture of Type I and Type III macromolecules in which the shape and dimension of tropocollagen in its natural helical orientation is retained. Semed S is free of telopeptides. Semed F is a water insoluble form of collagen fibers which may also be used in the final product form. Initial conversations with Semex revealed the following technical information relative to their collagen products: 1. Non-pyrogenic; 2. Non-antigen (clinically); 3. Total cumulative heavy metals < 50 ppm, Na and Ca higher; and 4. Amino acid analysis and HPLC routinely done to insure lot to lot reproducibility. This technical information will be confirmed by our testing plan, but it appears that this vendor will be suitable for the hemostatic agent envisioned.

2.1.2 Subtask 1.2: Protein Content Studies

Initial gel electrophoresis experiments on the chitosan glutamate indicate the absence of significant amounts of high molecular weight protein. The detection limits for this experiment were 200 micrograms protein/gram chitosan glutamate. Further experiments in which possible protein contaminants will first be hydrolyzed to their constituent amino acids and then analyzed by HPLC will be conducted next quarter.

2.1.3 Subtask 1.3: Biodegradation Studies

Reagents have been received so that initiation of in vitro studies on the enzymatic (lysozyme) degradation of lyophilized chitosan glutamate can occur next quarter. In vivo studies await further developments in formulation and optimization of the product form (see Task 2).

2.2 Task 2: Formulation and Optimization

The hemostatic agent will be in the form of a lyophilized sponge. It will be a composite of collagen and chitosan glutamate. Preliminary experiments have indicated that sponges containing 30 weight% chitosan glutamate perform well as a hemostatic agent in the rat sagittal sinus bleeding model. Two parallel approaches are being taken in order to obtain the optimum sponge formulation, in terms of both handling characteristics and hemostatic activity. 3M has made sponges which have compositions ranging from 8 - 33% chitosan glutamate. The handling properties of these sponges are not yet satisfactory, but experimentation continues. 3M has also negotiated with Semex Medical which, in addition to being a source for collagen, is also rather expert in the technology of tray lyophilization. 3M visited Semex Medical which appears to be an excellent facility. All of their collagen experimentation is done under the guidelines of Good Manufacturing Practices which is a necessary requirement for the production of medical devices. Semex has agreed to make prototype sponges using chitosan glutamate and their collagen for our evaluation for both hemostatic activity and handling properties. The delivery of the first eight prototype sponges is expected yet this quarter. Since 3M anticipated locating an eventual manufacturer for the hemostatic sponge at some point, utilization of Semex Medical at this early stage will ensure a production facility for the samples necessary for preclinical and clinical testing. Testing of these sponges for hemostatic performance and histologic evaluation in rat subcutaneous and intraperitoneal implantation will commence next quarter.

2.3 Subtask 8.2: IDE Approval Process

3M met with members of the Office of Device Evaluation, FDA, in order to discuss the proposed testing plan for the hemostatic agent. This trip was planned so that the FDA could review our plan in the early stages and have input if 3M had overlooked any important details. The regulatory people at the meeting agreed that at this time an IDE for the product was appropriate, that is, that the hemostatic agent is a device and not a drug. There remains some question as to the extent of bioassay work which will be necessary. The original proposal calls for some of this type of work but not the complete line of genetic toxicity testing. It may be necessary to complete more toxicity testing on the final sponge product than is currently in the proposal. A definitive plan will evolve as results from initial testing become available. 3M plans to keep an open dialogue with the FDA relative to this issue so that a satisfactory IDE can be prepared as described in the proposal.

3.0 CONCLUSIONS

Research has begun on the 'Hemostatic Activity of Chitosan in Wound Management' project. Suitable sources for both the raw materials for the hemostatic agent have been located and characterization of the chitosan and collagen has been initiated. Prototype product is currently being made at both 3M and an outside vendor. Hemostatic activity and handling characteristics of these samples will be determined early next quarter.

4.0 REFERENCES

(1) Muzzarelli, R.A.A. and Rocchetti, R., "The Determination of the Degree of Acetylation of Chitosans by Spectrophotometry," in Chitin in Nature and Technology, Muzzarelli, R., Jeuniaux, C., and Gooday, G.W., Eds., Plenum Press, New York, NY, 1986.